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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/13, 15/11, 5/10, C07K 16/00, 16/85, 1/21, 1/42, A61K 39/395

A2

(11) International Publication Number: WO 97/30156

(43) International Publication Date: 21 August 1997 (21.08.97)

(21) International Application Number: PCT/US97/02322

(22) International Filing Date: 14 February 1997 (14.02.97)

(30) Priority Data:
08/601,197 14 February 1996 (14.02.96) US

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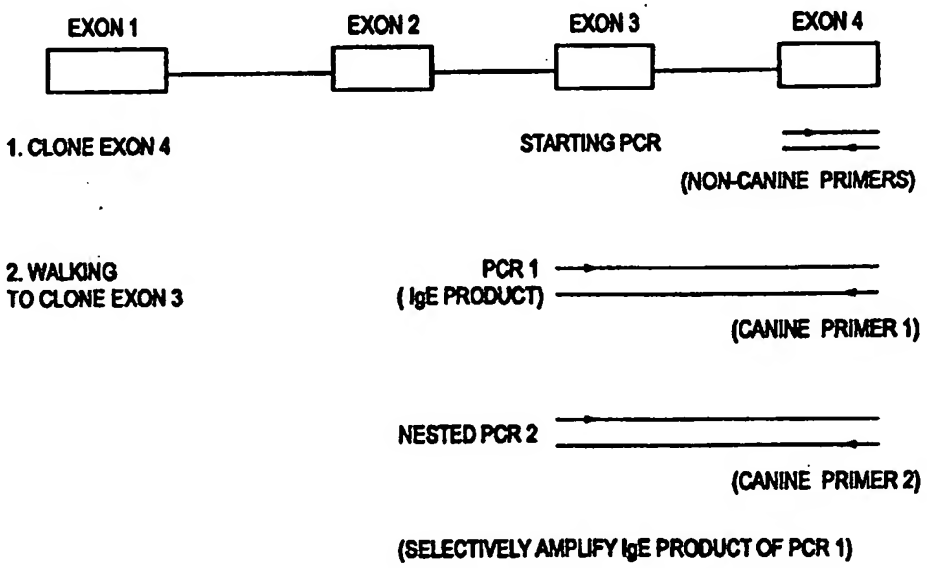
(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: NUCLEOTIDES AND PEPTIDES CORRESPONDING TO THE CANINE IgE HEAVY CHAIN CONSTANT REGION AND RELATED METHODS



(57) Abstract

Recombinant DNA molecules encoding complete canine IgE heavy chain constant region and the DNA sequence for all six exons of canine IgE are disclosed. The canine IgE heavy chain constant region DNA sequence was isolated using a nested walking procedure that is also disclosed. Also disclosed are peptides encoded by said sequences, including recombinant canine IgE heavy chain peptides produced by prokaryotic or eukaryotic cells. Such peptides are used in methods to treat the manifestation of allergy in dogs. Disclosed are antibodies that bind to peptides disclosed herein, as well as such antibodies for use to treat the manifestations of allergy in dogs.

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DESCRIPTION

Nucleotides and Peptides Corresponding to the Canine Ige Heavy Chain Constant Region and Related Methods

Cross-reference to Related Applications

This application is a continuation-in-part of U.S. application serial number 08/601,197 filed 14 February 1996 from which priority is claimed; this application is incorporated by reference herein.

Technical Field of the Invention

This application relates to the analysis and manipulation of genetic material. More specifically, the invention relates to recombinant DNA molecules encoding canine immunoglobulin proteins.

Background of the Invention

It is estimated that up to 30% of the canine population suffers from allergies and allergy-related skin disorders. Specifically, atopy (allergic dermatitis) has been estimated to affect between 3 and 15% of the entire population. The substances most likely to cause an allergic reaction vary from species to species. Common canine allergens include fleas, pollens, molds and dust. Given the prevalence of allergies in dogs, it has been desired to develop methods to properly diagnose and treat allergies in dogs.

Immunoglobulin E (hereinafter IgE) is a type of antibody that is understood to be an important component in mediating allergic responses, including Type I immediate hypersensi-tivity. IgE molecules bind to mast cells and basophils by Fc receptors on the cells. When such cell-bound IgE antibodies bind to an allergen, the allergen cross-links with other IgE antibodies on the cell surface. This cross-linking mediates Type I immediate hypersensitivity reactions by causing release of histamines and other molecules that produce symptoms associated with allergy.

Detection and quantitation of IgE antibodies is important in the diagnosis of Type 1 (immediate-type) hypersensitivity disease. Currently, it is understood that canine IgE is detected by several commercially available *in vitro* allergy tests. Such tests were designed to detect canine IgE in a sample, by contacting the sample with immobilized allergen, then any IgE bound to the immobilized allergen is detected with a polyclonal antibody understood to react with IgE. It is not clear, however, that the polyclonal antibodies react exclusively with IgE since the immunogens used to prepare the polyclonal antibodies were partially purified, native, glycosylated immunoglobulins. Polyclonal antibodies to such immunogens are likely to detect non-IgE immunoglob-ulins with anti-allergen specificity, since the other immunoglobulins likely contaminated the immunogen. In addition, antibodies directed against antibody

glycosylations are likely to cross-react with glycosylations on IgE and non-IgE immunoglobulins.

The sequences for the genes encoding human and murine IgE heavy chain constant region are known (For example, Ishida et al., "The Nucleotide Sequence of the Mouse Immunoglobulin E Gene: Comparison with the Human Epsilon Gene Sequence", EMBO Journal 1:1117-1123 (1982). The human and murine genes possess 60% homology within exons, and 45-50% homology within introns, with various insertions and deletions. Subsequent to the present invention, Patel et al. published the nucleotide and predicted amino acid sequence for exons 1-4 of the heavy chain constant region of canine IgE in the article "Sequence of the Dog Immunoglobulin Alpha and Epsilon Constant Region Genes," Immunogenetics 41:282-286 (22 March 1995). The complete sequence of the canine IgE heavy chain constant region, with membrane bound portions encoded by exons 5 and 6, is disclosed for the first time herein.

Summary of the Invention

The present invention relates to recombinant DNA molecules, and conservative variants thereof, that encode elements of the structural gene for canine IgE. The complete nucleotide sequence that encodes the canine IgE heavy chain constant region (epsilon) is disclosed for the first time herein.

In one aspect of the present invention a recombinant DNA molecule, and conservative variants thereof,

comprising DNA sequence of exons one, two, three, four, five, or six of the canine IgE heavy chain constant region molecule is set forth.

In another aspect of the present invention, recombinant vectors, such as expression vectors, comprising a DNA sequence encoding canine IgE heavy chain constant region or components thereof are set forth.

In another aspect of the present invention, polypeptides produced using a recombinant expression vector containing a canine IgE heavy chain DNA sequence are set forth.

In another aspect of the invention, compositions comprising a nucleotide sequence that is antisense to a DNA sequence encoding a component of canine IgE heavy chain is set forth; the component can be the full IgE heavy chain.

Disclosed is a cloning vector comprising a DNA sequence of canine IgE genomic material; a recombinant cell line comprising a DNA sequence of canine IgE genomic material; a method for producing a polypeptide comprising a step of expressing peptide comprising an amino acid sequence encoded by a cloning vector comprising canine IgE genomic material; a cell that expresses a recombinant polypeptide encoded by a DNA sequence (or conservative variants thereof) that encodes canine IgE heavy chain constant region or components thereof.

Accordingly, an object of the present invention is to provide the full DNA sequence for the canine IgE constant region molecule.

Brief Description of The Drawings

Figure 1 is a schematic outline of the nested walking procedure used for the initial cloning of the exons 1-4.

Figure 2 depicts a comparison of cysteine conservation between a portion (exons 1-4) of the sequence encoding canine IgE heavy chain isolated herein (Seq 3 in Fig. 2), compared to portions of murine (Seq 2 in Fig. 2) and human (Seq 1 in Fig. 2) sequences encoding IgE heavy chain constant regions; (*) indicates cysteine, the arrow indicates the onset of canine exon 4.

Figure 3 shows the DNA sequence and predicted amino acid sequence of exons 1-4, and surrounding noncoding DNA of the sequence encoding canine IgE heavy chain constant region. N = unknown nucleotide; Xaa = unknown amino acid.

Figure 4 shows the DNA sequence and predicted amino acid sequence of exons 5 and 6, and surrounding noncoding DNA, of the canine DNA sequence encoding the IgE heavy chain constant region. The probable beginning of exons 5 and 6 are shown.

Figure 5 shows the nucleic acid and amino acid sequences for the signal (secretion) sequence utilized to obtain expression of selected nucleic acid sequences of canine IgE DNA in eukaryotic cells, ligated with canine IgE heavy chain exons 2-4. Upon creation of a recombinant nucleic acid sequence containing the signal sequence, recombinant peptides having functions of native canine IgE were expressed in eukaryotic cells without, however, the need for nucleic acid sequences encoding the entirety of

IgE heavy chain constant and variable regions as well as the light chains.

Detailed Description of The Invention

Definitions

cDNA clone: A duplex DNA sequence representing an RNA, carried in a cloning vector.

Cloning: The selection and propagation of a single DNA species.

Cloning Vector: A plasmid, phage DNA or other DNA sequences, able to replicate in a host cell and capable of carrying exogenously added DNA sequence for purposes of amplification or expression of the added DNA sequence.

Codon: A triplet of nucleotides that represents an amino acid or termination signal.

DNA Sequence: A linear series of nucleotides connected one to the other by phosphodiester bonds between the 3' and 5' carbons of adjacent pentoses.

Expression: The process undergone by a structural gene to produce a polypeptide. It is a combination of transcription and translation.

Expression Control Sequence: A DNA sequence of nucleotides that controls and regulates expression of structural genes when operatively linked to those genes.

Exon: A contiguous region of DNA encoding a portion of a polypeptide. Reference to any exon, e.g. "DNA sequence of exon 6", refers to the complete exon or any portion thereof.

Genome: The entire DNA of a substance. It includes inter alia the structural genes encoding for the polypeptides of the substance, as well as operator, promotor and ribosome binding and interaction sequences such as the Shine-Dalgarno sequences.

Nucleotide: A monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The four DNA bases are adenine ("A"), guanine ("G"), cytosine ("C") and thymine ("T"). The four RNA bases are A, G, C and uracil ("U"). A and G are purines, and C, T, and U are pyrimidines.

Phage or Bacteriophage: Bacterial virus many of which include DNA sequences encapsidated in a protein envelope or coat ("capsid").

Plasmid: An autonomous self-replicating extrachromosomal circular DNA.

Polymerase Chain Reaction (PCR): A method of amplifying a target DNA sequence contained in a mixture of DNA sequences, by using oligonucleotide primers that flank the target DNA sequence for repeated cycles of DNA synthesis of the target DNA sequence.

Polypeptide: A linear series of amino acids connected one to the other by peptide bonds between the α-amino and carboxy groups of adjacent amino acids.

Reading Frame: The grouping of codons during translation of mRNA into amino acid sequences. For example, the sequence GCTGGTGTAAG may be translated in three reading frames or phases, each of which affords a different amino acid sequence:

GCT GGT TGT AAG-Ala-Gly-Cys-Lys

G CTG GTT GTA AG-Leu-Val-Val

GC TGG TTG TAA A-Trp-Leu-

(STOP).

Recombinant DNA Molecule: A hybrid DNA sequence comprising at least two nucleotide sequences, the first sequence not normally being found together in nature with the second.

Structural Gene: A DNA sequence which encodes through its template or messenger RNA ("mRNA") a sequence of amino acids characteristic of a specific polypeptide.

Transcription: Synthesis of RNA on a DNA template.

Translation: Synthesis of protein on the mRNA template.

Initial Cloning of Canine DNA Corresponding to Exons 1-4 of the IgE Heavy Chain Constant Region

The most direct method of isolating a specific DNA fragment from a mixture of recombinant DNA clones is to use a DNA probe derived from a known target DNA sequence. Prior to the present invention, however, canine IgE DNA was not available. In such situations a number of methods in the art are used to isolate a gene from one species, based on homology with the corresponding gene of known sequence from another species. Typically, DNA fragments derived from homologous genes from more distantly related species, show some small regions of greater homology scattered among regions of much lower homology. The more homologous segments form the basis for interspecies

identification with such probes. Therefore, DNA derived from the IgE gene from at least one other species is used. In order for this method to be practical, however, sufficient homology must be found between the target DNA and the probe. If homology is not sufficient, it will not be possible to distinguish binding of probe to target from nonspecific binding. It was known that there was a low degree of homology between mouse and human IgE heavy chain constant region genes. Accordingly, a low degree of homology between canine IgE heavy chain constant region genes and the genes of these other species was also expected; this low degree of homology made the use of prior art methods difficult.

Accordingly, in an effort to clone the gene encoding the canine IgE heavy chain constant region (epsilon), a method based on polymerase chain reaction (PCR) amplification was developed. A PCR-based "walking" approach was used on canine genomic DNA isolated from whole blood (Promega, Inc.). Although less direct than having a known probe sequence, PCR using related oligonucleotides has several advantages over direct DNA probe methods. First, it is more practical with PCR to employ a spectrum of different physical parameters such as temperature in order to allow specific binding to target DNA. Second, the size of the resulting PCR product shows that not only does a single gene contain homology with two oligonucleotide primers, but their relative locations are as predicted. The combination of these factors makes it

more likely that PCR will successfully locate DNA fragments based on partially homologous oligonucleotides.

The PCR walking approach does not guarantee that interspecies homology will effectively allow isolation of homologous DNA fragments. There is reliance on primers derived from other species; thus, if the homology with such other species is insufficient, no target fragments will be obtained.

In the present cloning of DNA encoding canine IgE heavy chain constant region, a step was introduced in an effort to insure that authentic canine IgE-encoding fragments were isolated by PCR. The step was based on the concepts of "genomic walking" and of nested PCR. Genomic walking refers to isolation of a DNA fragment prepared from the end of a starting DNA, and its subsequent use as a probe to isolate an overlapping cloned DNA fragment which also contains the probe region. Nested PCR is a technique designed to increase the specificity of PCR amplification. With nested PCR, the products of an initial PCR are reamplified with a second set of primers selected from within the specific predicted product. Only the specific product, and not any unrelated products, should be amplified in the second reaction. Nested PCR is commonly used when the target DNA is present at low levels in a starting sample likely to contain many non-specific targets. In such situations, a relatively high contribution of non-specific products in the final set of products is expected. A second, nested PCR, should greatly increase the proportion of specific products. In

the typical nested PCR situation, however, the sequence of the target is known, and only primers which match that target are used.

To adapt the genomic walking concept to PCR, and to use the method to isolate fragments based on interspecies homology, an oligonucleotide from one end of a cloned fragment was used as a primer; a second primer was determined based on the possibility of interspecies homology (rather than being derived from a known sequence from the same species as with usual nested PCR). To enhance the specificity of the present approach, a second PCR reaction was performed. The products of the first reaction were amplified substituting a different primer obtained from within the starting cloned fragment. Only one of the primers in the second PCR was specific for the authentic product, unlike the common case with nested PCR where all primers are specific for a known product.

The PCR-based "walking" approach employed herein is depicted in Figure 1. This walking approach was used to clone, consecutively, genomic fragments containing copies of exons 4, 3, 2, and 1, including the intervening sequences separating them.

This PCR-based method was carried out as follows: One end of the target gene (exon 4 in this case) was cloned by PCR with a primer pair selected from either a murine or a human IgE heavy chain constant region-encoding sequence. These PCR primers were selected from regions which demonstrated relatively high sequence conservation between the murine and human sequences.

An initial fragment of canine exon 4 was obtained after cloning and sequencing products from a variety of different PCR reactions with different primer pairs. The fragment was determined to be canine exon 4 based on comparison of DNA and amino acid sequences between murine and human exon 4. Although the degree of homology was low, the pattern of conserved residues was similar among the three sequences (Fig. 2). Once the initial canine fragment of exon 4 was sequenced, additional clones containing adjacent DNA were isolated by the nested PCR-based "walking" approach.

To obtain clones of adjacent exon 3 (and intron 3), a PCR (referred to as PCR1) was performed with one primer selected from the mouse or human exon 3 sequences and with a second primer from within the cloned canine exon 4 sequence. In PCR1 at least one primer would be a match with the target, the primer derived from the authentic canine exon 4 sequence. Since one of the primers was likely not to be a precise match, the products of the PCR reaction include a set of "false" products detected as an unresolved "smear", presumably containing the authentic product.

To amplify the authentic product but not the false products and to extend the cloned DNA into the adjacent exon 3 (and intron 3), a second nested PCR reaction with a different canine primer was performed using the products of the PCR1 as the targets. In this particular case, the primers included the non-canine exon 3 primer which was used in PCR1, and a second authentic canine primer from

within exon 4. Among the products of the first reaction, only the authentic IgE heavy chain constant region product contained exon 4 and therefore was amplified in the second PCR. The fragment resulting was cloned and sequenced.

This nested "walking" procedure was used to clone, consecutively, genomic fragments containing copies of exons 4, 3, 2 and 1, including the intervening sequences separating them. Nested PCR reactions which involved one "authentic" canine primer were performed under conditions of higher stringency, so that fewer DNA sequences were modified and so that the sequences modified more closely correspond to the primers.

The PCR products were cloned in the plasmid pUC19 (Boehringer-Mannheim), and the DNA sequence was determined. Sequence comparison with the human and mouse sequences indicated that the product represented canine IgE heavy chain constant region, since even though the homology was low, when there was a sequence match between two of the species, the match often tended to occur in the genes of each of mouse, human and dog. For example, a comparison of cysteine conservation, an important amino acid in immunoglobulin structure, is depicted in Figure 2.

The PCR primers used herein are indicated in Table 1.

Table 1

IgE heavy chain constant region exon, plasmid (plasmid synonyms)	PCR	PCR primers
exon 4 19IgE (19IgE-4)		TGATCCAGAACTTCATGCCTGAGGA AGGCGACTGAAGATGAAGAAGCC
exon 3 19IgE3/4	PCR 1	CCTACCTAAGCCGGCCCAGCCCGTTCGACCTGTT AGGCGACTGAAGATGAAGAAGCC
	Nested PCR 2	CCTACCTAAGCCGGCCCAGCCCGTTCGACCTGTT CCGTGGTGGTGTACTGGTCT
exon 2 19ce23	PCR 1	AGAAGCACTGGCTGTCAGACCGCACCTACACCTG TAGACGTGACTGTGATCGTCC
	Nested PCR 2	AGAAGCACTGGCTGTCAGACCGCACCTACACCTG CCGGTACCAGGTCAGGTTCA
exon 1 19ce12A-1	PCR 1	TGACTCTGGGCTGCCTGGCCACGGGCTACTCCC GGCCATACCTGAGCACTTGCG
	Nested PCR 2	TGACTCTGGGCTGCCTGGCCACGGGCTACTCCC CTGAGCACTTGCGAGCCTCAT

It will be appreciated by one skilled in the art that primers other than those set forth here are also effective in the isolation and amplification DNA encoding canine IgE heavy chain constant region in accordance with the methodology disclosed herein.

It will be appreciated by one of ordinary skill in the art that a wide variety of host/cloning vector combinations are usefully employed in cloning the DNA isolated as above. For example, useful cloning vehicles/vectors have included pMALc2 (New England Biolabs), and pGST (Pharmacia) to express canine IgE

fragments in bacteria; the canine IgE DNA has been propagated in pUC19 (Boehringer-Mannheim) and λZAP (Stratagene). Cloning vehicles comprise various known bacterial plasmids such as pBR322, other *E. coli* plasmids and their derivatives and wider host range plasmids such as RP4; phage DNA such as the numerous derivatives of phage λ, *e.g.*, NB989; and, vectors derived from a combination of plasmid and phage DNA, such as plasmids which have been modified to employ phage DNA expression control sequences. Vectors which replicate in eukaryotic cells can also be used. Useful hosts comprise bacterial hosts such as *E. coli* strains X1776, X2282, HB101 and MRC1; strains of *Pseudomonas*, *Bacillus subtilis* and other bacilli; yeasts and other fungi; animal or plant hosts such as animal or plant cells in culture; and other hosts. As appreciated by one of ordinary skill in the art, not all hosts are equally efficient. The particular selection of host-cloning vehicle combination may be made by those of skill in the art after due consideration of the principles set forth herein without departing from the scope of this invention.

Isolation of Genomic Clones Containing Exons 1-6 of Canine IgE Heavy Chain Constant Region DNA

In order to obtain the complete gene for the canine IgE heavy chain constant region, a canine genomic DNA library was prepared and screened using the λZAP vector. Genomic DNA was digested with the restriction endonuclease BamHI. A probe was prepared from canine exon 1, cloned as

described herein; this probe was used to screen the library. A 7 kB canine genomic fragment, clone cε7, was isolated and characterized; the plasmid pBKcε7 contained the genomic material of subclone pBKcε7; pBKcε7 was constructed from the λZAP clone by in vivo excision. DNA sequencing indicated that clone cε7 included exons 1, 2, 3 and part of exon 4 extending through a BamHI site near the end of exon 4. It was determined that BamHI had digested near the end of exon 4 locus based on comparisons with murine and human exon 4, and the absence of a termination codon.

In order to isolate a clone containing the remainder of the canine IgE heavy chain constant region locus, a second genomic library was constructed with genomic DNA after digestion with restriction endonuclease HindIII and the library was screened with the probe prepared from exon 1 which was used in the isolation of the clone cε7. Clone cε5, which resulted, was a 5 kB fragment which contained exons 1, 2, 3, 4, 5, and 6, and a large intron separating exons 5 and 6 from the others; plasmid pBKcε5 which was constructed by *in vivo* excision contained the genomic material of clone cε5. Exons 5 and 6 are believed to encode additional amino acid residues specifically found in the cell-associated form of IgE but not in the secreted form.

Figure 3 shows the DNA sequence and predicted amino acid sequence of exons 1-4 and surrounding noncoding DNA of canine IgE heavy chain constant region. As discussed in greater detail herein, the exon boundaries were

determined based on sequencing cloned cDNA copies of mRNA isolated from canine lymphocytes as discussed herein. The exon boundaries are represented in Fig. 3 (SEQ ID NO: 1) as follows: the DNA sequence of exon 1, nucleotides 167-448; the DNA sequence of exon 2, nucleotides 608-931; the DNA sequence of exon 3, nucleotides 1024-1344; and, the DNA sequence of exon 4, nucleotides 1419-1742. Underlined nucleotides 1835-1840 show the polyadenylation signal for the secreted form.

The complete DNA sequence of the locus containing exons 5 and 6 is indicated in Figure 4. Figure 4 also shows the predicted amino acid sequence of exons 5 and 6, and surrounding noncoding DNA near the canine IgE DNA sequence. The beginnings of exons 5 and 6 as presently understood are shown in Fig. 4; the beginning sites were based on comparisons with human sequences, and not on cDNA sequences; the end of each exon was determined based on general sequence motifs known to those of skill in the art to be found at the ends of exons. The precise limits of the exons is known upon examination of the mRNA with the exons spliced together. The exon boundaries, as determined, are depicted in Fig. 4 (SEQ ID NO: 2) as follows: the DNA sequence of exon 5, nucleotides 82-216; and, the DNA sequence of exon 6, nucleotides 316-390.

Preparation of Canine Genomic DNA

A canine genomic DNA fragment comprising IgE heavy chain epsilon exons, or components thereof, is available is deposited with the ATCC as Accession Numbers (not yet

assigned). Alternatively, a canine genomic DNA fragment comprising IgE epsilon exons, or components thereof, is obtained by isolation of the 5 kilobase HindIII fragment referred to herein as ce5, or portions thereof. To isolate canine genomic DNA corresponding to the sequence of ce5, a library of 5 kilobase fragments is prepared from canine genomic DNA digested with restriction endonuclease HindIII; digestion with this endonuclease was determined to contain complete canine heavy chain constant region exons 1-6 in the section "Isolation of Genomic Clones Containing Exons 1-6 of Canine IgE Heavy Chain Constant Region DNA" herein. The library is screened with a DNA probe. To prepare the probe, PCR is performed with canine genomic DNA as target, with primers constructed in accordance with standard methodologies to amplify a fragment between coordinates 251 and 444 as defined in Fig. 3(SEQ ID No. 1). The sequence of HindIII digested DNA identified with the probe is isolated.

Characterization of Messenger RNA for Canine IgE Heavy Chain Constant Region

As indicated herein, sequence comparisons using the deduced amino acid sequence of the cloned DNA showed patterns of conservation consistent with a determination that the canine IgE Heavy chain epsilon sequence was isolated. However, it was possible that the cloned DNA represented a nonfunctional canine gene. To rule out the possibility that the cloned DNA was for a nonfunctional gene, analysis for the presence of mRNA copies of the gene

in canine lymphocytes was performed. To determine whether lymphocytes contained such mRNA copies, cDNA cloning and sequencing was used. Purified mRNA from canine lymphocytes was converted into DNA and PCR was performed with primers based on the cloned genomic sequence. This protocol was carried out in accordance with the principle that isolation of PCR products whose sequence matched the canine genomic exon sequences would, therefore, indicate that the cloned gene was expressed in dog lymphocytes. Canine lymphocytes were isolated from canine blood and mRNA was purified using standard procedures. The mRNA was converted to double stranded cDNA by reverse transcription and PCR amplification with primers based on the genomic DNA sequence, and cloned into pUC19. Several cDNA clones were identified and sequenced. Such cDNA cloning of mRNA and subsequent sequencing was carried out for several cDNA clones. Sequencing of these clones was performed. The DNA sequence of the cDNA clones was as expected for transcripts of the genomic clone described above, confirming that the clone represented an active IgE heavy chain locus. The cDNA sequence also allowed assignment of the exon boundaries for exons 1, 2, 3, and 4, as indicated in Figure 4.

Expression of Recombinant IgE Heavy Chain Constant Region Proteins in *E. coli*.

A portion the DNA sequence encoding exon 3 was expressed as a recombinant fusion protein in *E. coli* by use of a cloning vector (in this context an expression

vector). The recombinant vector was a derivative of the commercially available plasmid pEX3 (Boehringer Mannheim). The plasmid pEX3 contained the bacteriophage lambda promoter/operator sequence and was designed to produce a fusion protein with β-galactosidase, which was encoded by the lacZ gene derived from *E. coli* with pEX3; expression was regulated by a temperature shift in the presence of the temperature sensitive cI857 repressor.

The pEX3 expression vector was modified by the deletion of 1885 nucleotides between the restriction enzyme sites EcoRV and SmaI in the β-galactosidase coding region. This deletion resulted in a deletion of about two-thirds of the β-galactosidase coding region, with and left sufficient DNA to encode for approximately 400 amino acids.

A portion of exon 3, encoding sixty-four amino acids, was ligated into the EcoRV/SmaI site of the modified pEX3 plasmid. The portion of exon 3 that was inserted corresponded to nucleotides 1081-1272 (as depicted, *e.g.*, in Fig. 3). The IgE DNA was inserted so that the reading frame of the encoded protein was in frame with the reading frame of the coding region for β-galactosidase, whereby a fusion protein between canine IgE constant region peptide and β-galactosidase was produced. The IgE fragment in plasmid 19IgE3/4 was oriented within vector pUC19 such that this translational fusion was generated by ligation of the 0.22kB EcoRI/SalI fragment from plasmid 19IgE3/4 with the 6.6kB EcoRI/SalI fragment from the modified pEX3 expression vector. The expression plasmid containing the

DNA from IgE constant region exon 3 was called ΔExce34 (the plasmid was also called deltaEXCH3, and the fusion protein produced was called the deltaEXCH3 protein).

Recombinant IgE plasmids ΔExce34 were introduced into *E. coli* strain N4830-1 by transformation (this cell line was called ΔExce34/*E. coli* N4830, and is deposited with the ATCC as Accession No.: (not yet assigned)). After growth of the bacteria overnight at 28°C, recombinant protein expression was induced by subculture into prewarmed medium at 42°C and incubation for 2 hours.

Purification of recombinant protein was accomplished using the following method. Induced cell pellets were sonicated successively on ice in the following solutions: TEN (0.1 M Tris-Cl, pH7.0, 10 mM EDTA, 0.15M NaCl); TET (50 mM Tris-Cl, pH7.5, 0.5 mM EDTA, 2% Triton X-100); TED (50 mM Tris-Cl, pH7.5, 0.5 mM EDTA, 2% sodium deoxycholate); TU (50 mM Tris-Cl, pH7.5, 2 M urea). After sonication in each solution, lysates were incubated 30 minutes on ice and insoluble recombinant proteins were sedimented by centrifugation at 10,000 x G for 10 minutes at 4°C. The final pellet was dissolved in TU9DTT (50mM Tris-Cl pH 7.5, 9M urea, 1 mM Dithiothreitol) by sonication at room temperature; was incubated 30 minutes with shaking at room temperature; and, the dissolved protein was clarified by centrifugation as above at room temperature. The supernatant was adjusted to 1 mg/ml and dialyzed against three changes of 100 volumes of 10 mM KPO_4 for three days. The purified protein was evaluated for purity by gel electrophoresis. The size of the

recombinant fusion protein was as predicted for a protein comprised of 64 amino acid residues of IgE C_H3 and the approximately 400 residues from β-galactosidase. Final proof that the protein contained canine IgE heavy chain constant region was determined based on cross-reactivity of monoclonal antibodies raised to authentic canine IgE with the recombinant protein.

One skilled in the art will appreciate that many different cloning vectors could be used to express canine IgE polypeptides, or to generate recombinant cell lines comprising canine IgE genomic material. The present example is an illustrative embodiment. The invention described herein comprises the cloning and cloning and expression of canine IgE heavy chain constant region material in various cloning and cloning-expression systems, including those derived from both prokaryotic or eukaryotic cells and organisms, those using other types of cloning vectors such as other plasmids, bacteriophage or viruses that replicate in eukaryotic cells.

Examples

The DNA sequence for canine IgE heavy chain constant region, as described herein, has been used to develop methods to facilitate the treatment of canine allergies, including Type I immediate-hypersensitivity reactions, see, *e.g.*, copending U.S. application Serial No. (not yet assigned), entitled Method and Compositions to Facilitate Treatment of Allergy in Dogs, filed 8 December 1995, in the name of MacKinnon et al, which is incorporated by

reference herein. The invention is also used to treat canine allergies by production of recombinant peptides, so as to achieve canine immunomodulation. These peptides are administered with art recognized pharmacologic excipients; these pharmacologic compositions are administered in pharmacologic dosages determined in accordance with skill known to those of ordinary skill in the art in view of the clinical presentation of the dog, to achieve canine immunomodulation.

Example 1: An antisense or a sense sequence to a DNA sequence encoding canine IgE heavy chain constant region is used to screen the genomic material of a non-canine species. Such screening identifies the region of the genome in the non-canine species that encodes IgE heavy chain constant region, where this IgE region is not yet known. In a preferred embodiment, the PCR walking approach of the present invention is employed to advantage in isolating IgE regions of a species.

Moreover, an antisense or a sense sequence to a canine IgE heavy chain constant region DNA sequence disclosed herein is used to determine the extent of homology between the canine IgE sequence and the sequence of an isolated noncanine IgE gene. Knowledge of interspecies homology, in combination with biological data, is used to locate functional regions in the IgE molecule of either species.

For example, it is determined that canine IgE and feline IgE both bind to the feline IgE receptor, thus the nucleic acid residues encoding the amino acids responsible for this binding would likely be found among those which

are homologous between the canine and feline sequences. Knowledge of the specific DNA sequence encoding the amino acids responsible for such binding is used to develop therapeutic compounds that inhibit the binding. For example, this is done by expressing a peptide comprising a binding domain which will compete with the binding of IgE to mast cells and basophils.

Additionally, it is determined that canine IgE but not feline IgE, bind to the canine IgE receptor. Once again, knowledge of interspecies sequence comparison is relevant. Pursuant to this knowledge of the extent of interspecies homology between dog and feline genomes, hybrid canine/feline IgE are developed. Various canine/feline IgE hybrids are analyzed to determine which bind to the canine receptor. The region of the canine sequence which encodes peptide involved in receptor binding is determined by comparison of the hybrids that do bind relative to those that do not bind to the canine receptor.

Example 2: Monoclonals were raised to a recombinant peptide comprising a component, preferably less than total, of canine IgE heavy chain constant region peptide. The monoclonals were screened to determine reactivity with native canine IgE. A monoclonal was found which was reactive with native canine IgE. Accordingly, this monoclonal was used to diagnose canine allergy in accordance with standard diagnostic techniques.

The two primary sources of nonspecificity present in the art by use of polyclonal serum to detect allergen-

specific canine IgE: 1) lack of specificity in detecting only IgE, and 2) the possibility of detecting any glycosylated antibody, are overcome by use of the present invention. These sources of nonspecificity were overcome by using a nonglycosylated component of IgE as a recombinant immunogen in order to develop monoclonal antibodies. Nonspecificity due to reactivity with any glycosylated antibody is overcome since when recombinant canine IgE is expressed in bacteria, it is nonglycosylated because bacteria do not glycosylate proteins.

Example 3: Heretofore, it has been believed that expression of canine IgE DNA in eukaryotic cells would require that the entire IgE molecule including the entire heavy chain constant region, as well as the heavy chain variable regions and the light chains. Although there have been reports that functional non-canine IgE peptides have been expressed, these reports have generally been anecdotal and no consistently reproducible protocols have been reported. No known functional canine IgE peptide has ever been reported. There is only one known report of expression of canine IgE DNA, wherein it was expressed as part of a chimeric mouse antibody. See, e.g., Chang, U.S. Patent 5,514,776, issued 7 May 1996.

As disclosed herein for the first time in the art, it has been found that canine IgE having less than the full constitution of light and heavy chains can be expressed in eukaryotic cells. Moreover, it has been found that proteins expressed thereby possess functional qualities of the canine IgE molecule. Accordingly, it is disclosed

that the region on the canine IgE molecule that is bound by the canine IgE receptor is contained within the peptide sequence encoded by exons CH2 through CH4.

A strategy for the rational design of therapeutic agents based on recombinant proteins is to develop a variant of a naturally occurring protein which retains some but not all of the properties of the authentic protein. For canine IgE, two relevant functions are its binding to the IgE receptor and its association with allergen. A variant recombinant IgE which retains the binding functions but can not associate with allergen is of therapeutic value within this context. In order for IgE to associate with allergen, the IgE variable domains are needed.

Based on the sequences disclosed herein, a recombinant protein was developed that lacked the ability to associate with allergen but possessed other native canine IgE characteristics. Accordingly, a recombinant protein derived from canine IgE regions CH2, CH3, and CH4 was developed. Recombinant DNA was designed and constructed so as to fuse a signal (secretion) sequence to canine IgE exons 2, 3, and 4. The signal secretion sequence is set forth in figure 5 together with canine IgE heavy chain sequences for exons 2-4. This recombinant DNA was inserted into a mammalian expression vector pcDNA3 (INVITROGEN, San Diego, CA) and the expression vector was introduced into mammalian cells (COS cells) by transfection. Supernatants from the transfected cells were evaluated by ELISA analysis and western blot. The

results indicated that the transfected cells produced a recombinant IgE which was secreted as a dimeric molecule. This secreted IgE polypeptide had an amino acid sequence encoded by canine IgE exons 2-4; this polypeptide was found to bind to a recombinant canine IgE receptor *in vitro*. Thus, it was determined that the canine IgE receptor binds a portion of the canine IgE molecule encoded by exons 2-4. The portion of the canine IgE molecule bound by the IgE receptor is herein termed the "Fc region." The truncated Fc region protein cannot associate with allergen because it lacks IgE variable regions. Since this truncated recombinant IgE protein cannot associate with allergen, it is used as an anti-allergy therapeutic agent. A recombinant IgE Fc region protein with identical properties was produced by insect cells infected with recombinant baculovirus, and is also used as an anti-allergy therapeutic agent.

Accordingly, a recombinant canine IgE molecule comprising protein as disclosed herein is produced that comprises a region corresponding to the Fc region of canine IgE while lacking the allergen-binding components of the native IgE molecule. This peptide is administered in a pharmaceutical form to a dog experiencing allergic disease. The dosage administered is modulated in accordance with skill held by one of ordinary skill in the art in view of the clinical manifestations of the patient. Such recombinant peptides bind to the surfaces of mast cells and basophils by the canine IgE Fc receptors on these cells. The binding of the recombinant peptides

competes with and precludes the binding of circulating canine IgE to the surfaces of these cells. Since the recombinant peptides do not have allergen-binding regions, allergen cannot be bound, and anaphylatoxin release is diminished.

Example 4: As described in Example 3, a recombinant canine IgE nucleic acid sequence corresponding to less than the complete canine IgE heavy chain constant and variable regions together with the canine IgE light chains was produced which led to a secreted recombinant IgE peptide fragment; this fragment retained several functions of the authentic IgE. Similarly, a recombinant IgE corresponding to: CH1, CH2, CH3, or CH4 (or any combination thereof); and, CH5 and/or CH6 was produced by mammalian cells transfected with the corresponding recombinant canine DNA. These cells are evaluated for the expression of cell-bound IgE. A cell-line expressing this truncated surface IgE is used to raise antibodies which bind to IgE on the surface of memory B-cells, monoclonal or polyclonal antibodies are raised for this purpose.

Accordingly, glycosylated (e.g., expressed in eukaryotic cells) recombinant canine IgE heavy chain epsilon protein is used to produce a canine antibody to achieve immunomodulation; monoclonal or polyclonal antibodies are used for this purpose. IgE-producing B-lymphocytes (memory B cells) have IgE molecules on their surfaces. The surface IgE is membrane bound due to amino acid residues encoded by exons 5 and 6. The amino acid residues encoded by exons 5 or 6 are not part of secreted

IgE heavy chain constant region which is completely encoded by exons 1, 2, 3 and 4. The amino acids encoded by exon 5 are not entirely encompassed by the membrane; a component of the peptide encoded by this exon is accessible in the extramembrane environment.

For example, a cell-line is developed that expresses recombinant IgE polypeptide comprising peptide encoded by exon 5 and/or exon 6. The polypeptide can optionally comprise peptide encoded by canine IgE heavy chain epsilon exons 1, 2, 3, 4, or 6. The membrane-bound peptide produced is used as an immunogen in the production, e.g., of monoclonal antibodies. The monoclonals raised to such immunogen are screened. Monoclonals that inhibit the binding of membrane-bound IgE to a material to which the bound IgE has binding affinity are chosen; these monoclonals are administered to achieve immunomodulation e.g. by lessening IgE production by memory B cells. Alternatively, monoclonals are chosen which are cytotoxic to memory B cells that express IgE.

Antibodies are chosen which bind to membrane-bound IgE not other IgE molecules to any adverse degree. Adverse binding to IgE populations which are not membrane-bound may lead to an undesired decrease in an animal's defense to parasitic organisms.

These monoclonals are administered to a dog experiencing allergic disease. These monoclonal antibodies treat allergic disease in dogs by decreasing the extent of Type I hypersensitivity reactions. These hypersensitivity reactions are diminished by lessening the binding of allergens to membrane-bound IgE on memory B

cells. Absent immunomodulation of this type, allergens would bind to membrane-bound IgE and this binding leading to production of circulating IgE that would bind to the surface of mast cells and basophils by Fc receptors on these cells, the Fc receptor-bound IgE would then bind to allergens leading to the release of anaphylaxis-inducing substances, such as vasoactive amines.

Example 5: A truncated recombinant IgE described in Example 3 and Example 4 is used to develop antibodies which react with authentic canine IgE. Bacterial-derived recombinant canine IgE peptides are also used. Accordingly, a recombinant canine IgE heavy chain constant region protein in accordance with the invention (glycosylated or nonglycosylated) is produced that comprises a peptide region corresponding to the Fc region of canine IgE. Monoclonal antibodies are raised to this peptide. A pharmaceutical is prepared which comprises such monoclonal antibodies and a pharmaceutical excipient. The pharmaceutical is administered to a dog experiencing allergic disease in a dosage determined by one skilled in the art based on the clinical manifestations of the dog. The monoclonal antibody binds to the Fc region of the dog's circulating IgE and prevents binding of the circulating IgE to mast cells or basophils which would have lead to type I hypersensitivity reactions.

Example 6: Scientific processes to develop variant peptides with improved (alternate) function include: By use of the sequence information disclosed herein

polypeptides are produced; one identifies peptide segments within said polypeptide which retain biological activity for some, but not all of the activity of the authentic polypeptide. In accordance with standard methodologies, and based on the sequence of such peptide segments, peptide analog libraries are developed which contain alterations at various locations within the peptide sequence. Screening of these libraries leads to identification of second generation peptides with improved or alternate function. (See, *e.g.*, Gordon EM, et al., "Applications of Combinatorial Technologies to Drug Discovery I.," *J. Med. Chem.* 37:1233-1251 (1994).)

Example 7: The sequence of the active peptides is utilized to design non-peptide analogs, often referred to as "small molecules." In accordance with methodologies known in the art, the chemical nature of the amino acid residues comprising the disclosed peptide or segments thereof is evaluated; this evaluation is used to determine organic compounds which contain analogous juxtapositioning of these chemical groups. (See, *e.g.*, Smith, R.G., et al. "A Nonpeptidyl Growth Hormone Secretogue," *Science* 260:1640 (1993).)

Example 8: Polypeptides in accordance with those disclosed herein are used to prepare a model that mimics the binding of canine IgE to IgE receptor; upon the disclosure herein, the model is prepared in accordance with methodologies known to those of ordinary skill in the art. This model is used to screen pharmaceutical compounds. Pursuant to this screening, compounds are

selected that have an affect on the extent of binding of IgE to receptor.

Example 9: Antisense reagents are oligonucleotides that are capable of entering a cell and binding to the sense mRNA. When the antisense reagents bind to the target sense mRNA, they block expression of the peptide encoded by the sense sequence. A further application of the sequence information disclosed herein comprises use of "antisense" reagents that specifically target cells which produce canine IgE messenger RNA. Antisense reagents based on the sequences of exons 1-4 target all IgE producing cells, and impair production of any IgE whether secreted or bound. Antisense reagents based on the sequences of exons 5 and 6 specifically target the memory B-cells that produce surface-bound IgE, and impair production of the bound IgE.

Closing

It will be appreciated by one skilled in the art that in addition to the DNA sequence for canine IgE heavy chain constant region disclosed herein, conservative variants are also effective for various utilities; accordingly, the invention comprises nontotal nucleotide sequences in accordance with those disclosed herein, as well as nucleotide sequences that have less than total homology but do exhibit specific hybridization with a sequence or segment thereof disclosed herein. Conservative variants include nucleotide substitutions that do not result in changes in the amino acid sequence as well as nucleotide

substitutions that result in conservative amino acid substitutions, or amino acid substitutions which do not substantially affect the character of the polypeptide translated from said nucleotides. For example, polypeptide character is not substantially affected if the substitutions do not preclude specific binding of the peptide to canine IgE receptor or other canine IgE ligands.

All publications mentioned herein are incorporated herein by reference to describe and disclose specific information for which the reference was thus discussed. It is to be noted that as used herein and in the appended claims, the singular forms "a" and "the" include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to "a formulation" includes mixtures of different formulations and reference to "the method of treatment" includes reference to equivalent steps and methods known to those skilled in the art, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skilled in the art. Although methods and materials or equivalent to those described herein can be used in the practice for testing of the invention, the preferred methods and materials are described herein. It is understood that the invention is limited solely be the appended claims.

Sequence Listing

(1) GENERAL INFORMATION

(i) APPLICANT: IDEXX Laboratories, Inc.

(ii) TITLE OF INVENTION: Recombinant DNA Molecules That Express Canine IgE Heavy Chain Constant Region and Components Thereof

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Lyon & Lyon

(B) STREET: 633 West Fifth Street

(C) CITY: Los Angeles

(D) STATE: California

(E) COUNTRY: USA

(F) ZIP: 90071-20066

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Consalvi, Mary S.

(B) REGISTRATION NUMBER: 32,212

(C) REFERENCE/DOCKET NUMBER: 213/223

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 213-489-1600

(B) TELEFAX: 213-955-0440

(2) INFORMATION FOR SEQUENCE ID NO: 1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 1842 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTCCAGTGAC CTCCATCTCT GCCCCCATGC TTTTCCTTCT 40
CAGACGCCCC CTGGGGCCAG GAGCAGGATA CCCCAGGTCA 80
ACAGCGGGCC TGGCATATGA TGGGGTGACA GTCCCAAGGC 120
AGGCACTGAC ACTGGNCCTG TCCCCACAGC CACCAGCCAG 160
GACCTG 166

TCT GTG TTC CCC TTG GCC TCC TGC TGT AAA GAC 199
Ser Val Phe Pro Leu Ala Ser Cys Cys Lys Asp

AAC ATC GCC AGT ACC TCT GTT ACA CTG GGC TGT 232
Asn Ile Ala Ser Thr Ser Val Thr Leu Gly Cys

CTG GTC ACC GGC TAT CTC CCC ATG TCG ACA ACT 265
Leu Val Thr Gly Tyr Leu Pro Met Ser Thr Thr

GTG ACC TGG GAC ACG GGG TCT CTA AAT AAG AAT 298
Val Thr Trp Asp Thr Gly Ser Leu Asn Lys Asn

GTC ACG ACC TTC CCC ACC ACC TTC CAC GAG ACC 331
Val Thr Thr Phe Pro Thr Thr Phe His Glu Tyr

TAC GGC CTC CAC AGC ATC GTC AGC CAG GTG ACC 364

Thr Gly Leu His Ser Ile Val Ser Gln Val Thr

GCC TCG GGC GAG TGG GCC AAA CAG AGG TTC ACC 397
Asp Ser Gly Glu Trp Ala Lys Gln Arg Phe Thr

TGC AGC GTG GCT CAC NNT GAG TCC ACC GCC ATC 430
Cys Ser Val Ala His Xaa Glu Ser Thr Ala Ile

AAC AAG ACC TTC AGT GGT 448
Asn Lys Thr Phe Ser Ala

AANCCAGGGT TNNNTGGCCA CATGACACTG GAGGGAGAAG 488
GGACAGGCTG GNGAATGCGC CATGGCTGGT AACGCCCAGC 528
ANATGTGGGG CTGGGGCTGA CACATGAGTC CCGTGGGCTN 568
AGAGACACCA CTGCCACATG GCTGCCTCTA CTTCTAGCA 607

TGT GCC TTA AAC TTC ATT CCG CCT ACC GTG AAG 640
Cys Ala Leu Asn Phe Ile Pro Pro Thr Val Lys

CTC TTC CAC TCC TCC TGC AAC CCC GTC GGT GAT 673
Leu Phe His Ser Ser Cys Asn Pro Val Gly Asp

ACC CAC ACC ACC ATC CAG CTC CTG TGC CTC ATC 706
Thr His Thr Thr Ile Gln Leu Leu Cys Leu Ile
0

TCT GGC TAC GTC CCA GGT GAC ATG GAG GTC ATC 739
Ser Gly Tyr Val Pro Gly Asp Met Glu Val Ile

TGG CTG GTG GAT GGG CAA AAG GCT ACA AAC ATA 772
Trp Leu Val Asp Gly Gln Lys Ala Thr Asn Ile

TTC CCA TAC ACT GCA CCC GGC ACA AAG GAG GGC 805
Phe Pro Tyr Thr Ala Pro Gly Thr Lys Glu Gly

AAC GTG ACC TCT ACC CAC AGC GAG CTC AAC ATC 838
Asn Val Thr Ser Thr His Ser Glu Leu Asn Ile

ACC CAG GGN NNG TGN GTA TCC CAA AAA ACC TAC 871
Thr Gln Gly Xaa Trp Val Ser Gln Lys Thr Tyr

ACC TGC CAG GTC ACC TAT CAA GGC TTT ACC TTT 904
Thr Cys Gln Val Thr Tyr Gln Gly Phe Thr Phe

AAA GAT GAG GCT CGC AAG TGC TCA GGT 931
Lys Asp Glu Ala Arg Lys Cys Ser Glu

ATGGCCCCCC TGTCCCCCAG AAACCCAGAT GCGCGAGGCT 971
CAGAGATGAG GGCCAAGGCA CGCCCTCATG CAGCCTCTCA 1011
CACACTGCAG AG 1023

TCC GAC CCC CGA GGC GTG AGC AGC TAC CTG AGC 1056
Asp Tyr Pro Arg Gly Val Ser Ser Tyr Leu Ser

CCA CCC AGC CCC CTT GAC CTG TAT GTC CAC AAG 1089
Pro Pro Ser Pro Leu Asp Leu Tyr Val His Lys

GCG CCC AAG ATC ACC TGC CTG GTA GTG GAC CTG 1122
Ala Pro Lys Ile Thr Cys Leu Val Val Asp Leu

GCC ACC ATG GAA GGC ATG AAC CTG ACC TGG TAC 1155
Ala Thr Met Glu Gly Met Asn Leu Thr Trp Tyr

CGG GAG AGC AAA GAA CCC GTG AAC CCG GTC CCT 1188

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TTG AAC AAG AAG GAT CAC TTC AAT GGG ACG ATC          1221
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ACA GTC ACG TCT ACC CTG CCA GTG AAC ACC AAT          1254
Thr Val Thr Ser Thr Leu Pro Val Asn Thr Asn

GAC TGG ATC GAG GGC GAG ACC TAC TAT TGC AGG          1287
Asp Trp Ile Glu Gly Glu Thr Tyr Tyr Cys Arg

GTG ACC CAC CCG CAC CTG CCC AAG GAC ATC GTG          1320
Val Thr His Pro His Leu Pro Lys Asp Ile Val

CGC TCC ATT GCC AAG GCC CCT GGT                     1344
Arg Ser Ile Ala Lys Ala Pro Gly

GAGCCACGGG CCCAGGGGAG GTGGGCGGGC CTCCTGANCC         1384
GGAGCCTGGG CTGACCCCAC ACCTATCCAC AGGC                1418

AAG CGT GCC CCC CCG GAT GTG TAC TTG TTC CTG          1451
Lys Arg Ala Pro Pro Asp Val Tyr Leu Phe Leu

CCA CCG GAG GAG GAG CAG GGG ACC AAG GAC AGA          1484
Pro Pro Glu Glu Glu Gln Gly Thr Lys Asp Arg

GTC ACC CTC ACG TGC CTG ATC CAG AAC TTC TTC          1517
Val Thr Leu Thr Cys Leu Ile Gln Asn Phe Phe

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Pro Glu Asp Ile Ser Val Gln Trp Leu Arg Asn
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GAC AGC CCC ATC CAG ACA GAC CAG TAC ACC ACC 1583
Asp Ser Pro Ile Gln Thr Asp Gln Tyr Thr Tyr

ACG GGG CCC CAC AAG GTC TCG GGC TCC AGG CCT 1616
Thr Gly Pro His Lys Val Ser Gly Ser Arg Pro

GCC TTC TTC ATC TTC AGT CGC CTG GTG GAC TGG 1649
Ala Phe Phe Ile Phe Ser Arg Leu Val Asp Trp

GAG CAG AAA AAC AAA TTC ACC TGC CAA GTG GTG 1682
Glu Gln Lys Asn Lys Phe Thr Cys Gln Val Val

CAT GAG GCG CTG TCC GGC TCT AGG ATC CTC CAG 1715
His Glu Ala Leu Ser Gly Ser Arg Ile Leu Gln

AAA TGG GTG TCC AAA ACC CCC GGT AAA 1742
Lys Trp Val Ser Lys Thr Pro Gly Lys

TGATGCCCAC CCTCCTCCCG CCGCCACCCC CCAGGGCTCC 1782
ACCTGCTGGG GCAGGGGAGG GGGGCTGGCA AGACCCTCCA 1822
TCTATCCTTN TCAATAAACA 1842

(2) INFORMATION FOR SEQUENCE ID NO: 2:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 538 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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A                                                          81

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Glu Leu Glu Leu Gln Glu Leu Cys Ala Asp Ala

ACT GAG AGT GAG GAG CTG GAC GAG CTG TGG GCC                147
Thr Glu Ser Glu Glu Leu Asp Glu Leu Trp Ala

AGC CTG CTC ATC TTC ATC ACC CTC TTC CTG CTC                180
Ser Leu Leu Ile Phe Ile Thr Leu Phe Leu Leu

AGA GTG AGC TAC GGC GCC ACC AGC ACC CTC TTC                213
Arg Val Ser Tyr Gly Ala Thr Ser Thr Leu Phe

AAG                                                        216
Lys

GTGGGCATGC AGAGCCCCTG GCCGGGGGTG GGGGCAGCAC                256
AGAGGGAGNG AGAGGTCCCG GCAGAGCTGT CCTCACATGT                296
GCCCTCCCCC CAGGTGAAG                                       315

TGG GTA CTC GCC ACC GTC CTG CAG GTG AAG CCA                348
Trp Val Leu Ala Thr Val Leu Gln Val Lys Pro

CAG GCC GCC CAA GAC TAC GCC AAC ATC GTG CGG                381
Gln Ala Ala Gln Asp Tyr Ala Asn Ile Val Arg

CCG GCA CAG                                                390
Pro Ala Gln
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CCCNNNNNCC GGCTGAGGGC AATCTGCTGG CCCTGAGTGG 470

GAGGAGGAAA GCAGACAAAC NCAGAGGGGC CAGAGCCAGA 510

CGCCCAGCAC ACACGGATCC AGAAGCTT 538

Claims

1. An isolated DNA sequence comprising: a DNA sequence of canine IgE heavy chain constant region exon 1 (SEQ ID NO: 1: nucleotides 167-448), or conservative variants thereof; a DNA sequence of canine IgE heavy chain constant region exon 2 (SEQ ID NO: 1: nucleotides 608-931), or conservative variants thereof; a DNA sequence of canine IgE heavy chain constant region exon 3 (SEQ ID NO: 1: nucleotides 1024-1344), or conservative variants thereof; a DNA sequence of canine IgE heavy chain constant region exon 4 (SEQ ID NO: 1: nucleotides 1419-1742), or conservative variants thereof; a DNA sequence of canine IgE heavy chain constant region exon 5 (SEQ ID NO: 2: nucleotides 82-216), or conservative variants thereof; or, a DNA sequence of canine IgE heavy chain constant region exon 6 (SEQ ID NO: 2: nucleotides 316-390), or conservative variants thereof.

2. A purified polypeptide comprising an amino acid sequence encoded by a nucleic acid sequence in accordance with claim 1.

3. A conservative variant of a polypeptide in accordance with claim 2.

4. A recombinant polypeptide of claim 2 produced by a eukaryotic cell.

5. A segment of a nucleotide sequence in accordance with claim 1 comprising eighteen contiguous nucleotides in a reading frame of: a DNA sequence of canine IgE heavy chain constant region exon 1 (SEQ ID NO: 1: nucleotides 167-448); a DNA sequence of canine IgE heavy chain constant region exon 2 (SEQ ID NO: 1: nucleotides 608-931); a DNA sequence of canine IgE heavy chain constant region exon 3 (SEQ ID NO: 1: nucleotides 1024-1344); a DNA sequence of canine IgE heavy chain constant region exon 4 (SEQ ID NO: 1: nucleotides 1419-1742); a DNA sequence of canine IgE heavy chain constant region exon 5 (SEQ ID NO: 2: nucleotides 82-216); or, a DNA sequence of canine IgE heavy chain constant region exon 6 (SEQ ID NO: 2: nucleotides 316-390).

6. A nucleotide sequence complementary to a sequence of claim 5.

7. A segment of a nucleotide sequence in accordance with claim 5 comprising twenty-one contiguous nucleotides.

8. A prokaryotic or eukaryotic cloning vector comprising a DNA sequence according to claim 1.

9. A prokaryotic vector of claim 8 wherein said vector is pBKce5 or Δexce34.

10. A eukaryotic vector of claim 8 wherein said vector expresses a polypeptide having a sequence encoded

by less than canine IgE heavy chain constant region exon 1 (SEQ ID NO: 1: nucleotides 167-448), or conservative variants thereof; the DNA sequence of canine IgE heavy chain constant region exon 2 (SEQ ID NO: 1: nucleotides 608-931), or conservative variants thereof; the DNA sequence of canine IgE heavy chain constant region exon 3 (SEQ ID NO: 1: nucleotides 1024-1344), or conservative variants thereof; the DNA sequence of canine IgE heavy chain constant region exon 4 (SEQ ID NO: 1: nucleotides 1419-1742), or conservative variants thereof; the DNA sequence of canine IgE heavy chain constant region exon 5 (SEQ ID NO: 2: nucleotides 82-216), or conservative variants thereof; and, the DNA sequence of canine IgE heavy chain constant region exon 6 (SEQ ID NO: 2: nucleotides 316-390), or conservative variants thereof.

11. A polypeptide encoded by a vector of claim 8.

12. A polypeptide encoded by a eukaryotic vector of claim 8.

13. A pharmaceutical composition comprising a polypeptide encoded by a vector of claim 8 and a pharmaceutical excipient.

14. An antibody raised to a polypeptide of claim 11.

15. An antibody of claim 14 for use in a method of treating the manifestations of allergy in a dog.

16. A pharmaceutical composition comprising an antibody of claim 14 and a pharmaceutical excipient.

17. A eukaryotic vector of claim 8 wherein said vector expresses a polypeptide encoded by the DNA sequence of canine IgE heavy chain constant region exon 2 (SEQ ID NO: 1: nucleotides 608-931), or conservative variants thereof; the DNA sequence of canine IgE heavy chain constant region exon 3 (SEQ ID NO: 1: nucleotides 1024-1344), or conservative variants thereof; and, the DNA sequence of canine IgE heavy chain constant region exon 4 (SEQ ID NO: 1: nucleotides 1419-1742), or conservative variants thereof.

18. A sequence of claim 1 which comprises a nucleic acid sequence which encodes the Fc region of the canine IgE molecule or conservative variants thereof.

19. The sequence of claim 18 which consists essentially of the DNA sequence of canine IgE heavy chain constant region exon 2 (SEQ ID NO: 1: nucleotides 608-931), or conservative variants thereof; the DNA sequence of canine IgE heavy chain constant region exon 3 (SEQ ID NO: 1: nucleotides 1024-1344), or conservative variants thereof; and, the DNA sequence of canine IgE heavy chain constant region exon 4 (SEQ ID NO: 1: nucleotides 1419-1742), or conservative variants thereof.

20. A polypeptide encoded by a sequence of claim 18.

21. A polypeptide of claim 20 for use in a method of treating allergic manifestations in a dog.

22. A recombinant cell line comprising a DNA sequence according to claim 1.

23. A recombinant eukaryotic cell line in accordance with claim 22.

24. A recombinant cell line in accordance with claim 22 wherein the cell line is ΔExcε34/*E. coli* 4830.

25. A method for producing a polypeptide having an amino acid sequence homologous to a component of canine IgE heavy chain constant region, said method comprising a step of expressing a peptide encoded by a cloning vector according to claim 8.

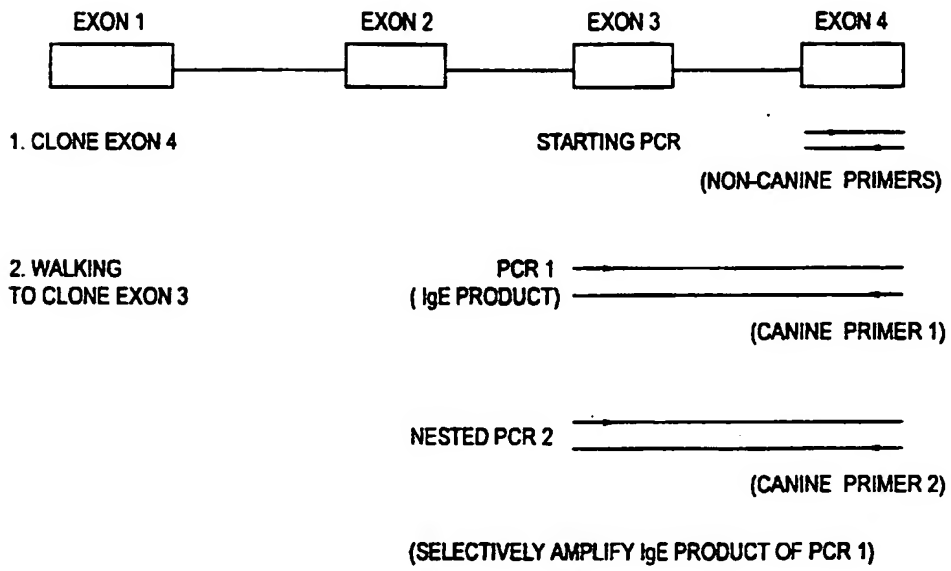
26. A cell that expresses a recombinant polypeptide encoded by a DNA sequence of claim 1.

27. An antisense reagent capable of binding to sense mRNA encoded by a nucleic acid sequence of claim 1; whereby upon binding of the antisense reagent to the sense mRNA, production of the peptide encoded by the mRNA is impaired.

28. The antisense reagent of claim 1 wherein the reagent impairs production of canine IgE.

29. The antisense reagent of claim 28 wherein the reagent impairs production of membrane-bound canine IgE.

Fig. 1 : NESTED WALKING PROCEDURE FOR CLONING CANINE IgE



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Seq 1 HHCO1J Heavy constant chain (IGE'CL') - Human
Seq 2 MHCO2X Heavy constant chain (IGE a 'CL) - Mouse
Seq 3 Translated sequence IDEXX IgE clone exons 1-4. spliced mRNA
           **          *
Seq 1 SVFPLTRCCKNIPSNATSVTLGCLATGYFPEPVMVTWDTGSLNGTTMTLPATTLTLSGHY
         ::   ::       : :::::   ::: :: ::: . ::: .:   ::    :
Seq 2    PLKP CKGTA    SMTLGCLVKDYFPGPVTVTWYSDSLNMSTVNFPALGSELK---
     :::::  :::      :::::::: ::: :    :::::::::    : : :     :
Seq 3 SVFPLASCCKD-NIASTSVTLGCLVTGYLPMSTTVTWDTGSLNKNVTTFPTTEHFTYGLH
                        *                                  *
Seq 1 ATISLLTVSGAWAKQMFTCRVAHTPSSTDW--VDNKTFSFTPPTVKILQSSCDGGGHFPP
       : : .:  :  ::. ::: : : ::  .   .   .    : ::. .: ::::   . :
Seq 2 VTTSQVTSWGKSAKN-FTCHVTHPPSFNESRTILVRPVNITEPTLELLHSSCDPNA-FHS
      .: .: :: :::: ::: :::  :.              : :::::.  :::    :
Seq 3 SIVSQVTASGEWAKQRFTCSVAHXESTAINKTFSACALNFIPPTVKLFHSSCNPVGDTHT
         *
Seq 1 TIQLLCLVSGYTPGTINITWLEDGQVMDVDLS-TASTTQEGELASTQSELTLSQKHWLSD
      :::: : . :      . ..:: :       :  :     :: :::: : : ..    : :.
Seq 2 TIQLYCFIYGHILNDVSVSWLMDDREITDTLAQTVLIKEEGKLASTCSKLNITEQQWMSE
      :::::::.::: ::    . :: :::         ::  : :: . :: ::: ..:   .:
Seq 3 TIQLLCLISGYVPGDMEVIWLVDGQKATNIFPYTAPGTKEGNVTSTHSELNITQXXXVSQ
        *          *                                  *
Seq 1 RTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSK
      :.:: :: ::   .   :..: :  ::::  ::  ::: ::.     : .:::::::    :
Seq 2 STFTCKVTSQGVDYLAHTRRCPDHEPRGVITYLIPPSPLDLYQNGAPKLTCLVVDLESEK
      .:::::::::: :: :  .:: .: ::::: ::: ::: ::.. : : :::::::::
Seq 3 KTYTCQVTYQGFTFKDEARKCSESDPRGVSSYLSPPSPLDLYVHKAPKITCLVVDLATME
                                                      *
Seq 1 GTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPR
        ::.::       :  :        :.: ..:: :::  .:::::  ::: : :: :.
Seq 2 N-VNVTWNQEKKTSVSASQWYTKHHNNATTSITSILPVVAKDWIEGYGYQCIVDHPDFPK
      :  :::: : :  :::          :::.::::::: : :::::::: :::::::::.
Seq 3 G-MNLTWYRESKEPVNPVPLNKKDHFNGTITVTSTLPVNTNDWIEGETYYCRVTHPHLPK
                                                    *
Seq 1 ALMRSTTKTSGPVGPRAAPEVYAFATP EWPGSRDKRTLACLIQNFMPEDISVQWLHNEV
      . :: ::: :    : ::::: :  : :   : ::::: :::::: :::::::::
Seq 2 PIVRSITKTPGQ---RSAPEVYVFPPPEEE  SEDKRTLTCLIQNFFPEDISVQWLGDGK
      . ::  :  :    :: :.:: :  : :  :..:. :: :::::: ::::::::: :.
Seq 3 DIVRSIAKAPGK---RAPPDVYLFLPPEEEQGTKDRVTLTCLIQNFFPEDISVQWLRNDS
                      ⟶ exon 4              *
Seq 1 QLPDARHSTTQPRKTKGS--GFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVS
      .    :::: : :. ::  :::.:::::: .  : :.  : :  .:::       . . .:
Seq 2 LISNSQHSTTTPLKSNGSNQGFFIFSRLEVAKTLWTQRKQFTCQVIHEALQKPRKLEKTIS
      .     .:: : :  ::   ::.:::::     .::::  : :  :::: : :  .:. ::
Seq 3 PIQTDQYTTTGPHKVSGSRPAFFIFSRL----VDWEQKNKFTCQVVHEALSGSRILQKWVS

Seq 1 VNPGK
```

FIG. 2A

```
          :
Seq 2 TSLGN
          :::
Seq 3 KTPGK
```

Seq 1	1-420	Seq 1	1-420
Seq 2	1-407	Seq 3	1-417
Aligned	422	Aligned	426
Matches	185	Matches	228
Mismatches	237	Mismatches	198
Homology	43%	Homology	53%

FIG. 2B

FIG. 3A. CANINE IgE HEAVY CHAIN CONSTANT CHAIN DNA SEQUENCE WITH TRANSLATED EXONS 1 THRU 4

```
GTCCAGTGAC CTCCATCTCT GCCCCCATGC TTTTCCTTCT                40
CAGACGCCCC CTGGGGCCAG GAGCAGGATA CCCCAGGTCA                80
ACAGCGGGCC TGGCATATGA TGGGGTGACA GTCCCAAGGC               120
AGGCACTGAC ACTGGNCCTG TCCCCACAGC CACCAGCCAG               160
GACCTG                                                   166

TCT GTG TTC CCC TTG GCC TCC TGC TGT AAA GAC               199
Ser Val Phe Pro Leu Ala Ser Cys Cys Lys Asp

AAC ATC GCC AGT ACC TCT GTT ACA CTG GGC TGT               232
Asn Ile Ala Ser Thr Ser Val Thr Leu Gly Cys

CTG GTC ACC GGC TAT CTC CCC ATG TCG ACA ACT               265
Leu Val Thr Gly Tyr Leu Pro Met Ser Thr Thr

GTG ACC TGG GAC ACG GGG TCT CTA AAT AAG AAT               298
Val Thr Trp Asp Thr Gly Ser Leu Asn Lys Asn

GTC ACG ACC TTC CCC ACC ACC TTC CAC GAG ACC               331
Val Thr Thr Phe Pro Thr Thr Phe His Glu Tyr

TAC GGC CTC CAC AGC ATC GTC AGC CAG GTG ACC               364
Thr Gly Leu His Ser Ile Val Ser Gln Val Thr

GCC TCG GGC GAG TGG GCC AAA CAG AGG TTC ACC               397
Asp Ser Gly Glu Trp Ala Lys Gln Arg Phe Thr

TGC AGC GTG GCT CAC NNT GAG TCC ACC GCC ATC               430
Cys Ser Val Ala His Xaa Glu Ser Thr Ala Ile

AAC AAG ACC TTC AGT GGT                                  448
Asn Lys Thr Phe Ser Ala

AANCCAGGGT TNNNTGGCCA CATGACACTG GAGGGAGAAG              488
GGACAGGCTG GNGAATGCGC CATGGCTGGT AACGCCCAGC              528

ANATGTGGGG CTGGGGCTGA CACATGAGTC CCGTGGGCTN              568
AGAGACACCA CTGCCACATG GCTGCCTCTA CTTCTAGCA                607

TGT GCC TTA AAC TTC ATT CCG CCT ACC GTG AAG               640
Cys Ala Leu Asn Phe Ile Pro Pro Thr Val Lys

CTC TTC CAC TCC TCC TGC AAC CCC GTC GGT GAT               673
Leu Phe His Ser Ser Cys Asn Pro Val Gly Asp
```

FIG. 3A

```
ACC CAC ACC ACC ATC CAG CTC CTG TGC CTC ATC                    706
Thr His Thr Thr Ile Gln Leu Leu Cys Leu Ile

TCT GGC TAC GTC CCA GGT GAC ATG GAG GTC ATC                    739
Ser Gly Tyr Val Pro Gly Asp Met Glu Val Ile

TGG CTG GTG GAT GGG CAA AAG GCT ACA AAC ATA                    772
Trp Leu Val Asp Gly Gln Lys Ala Thr Asn Ile

TTC CCA TAC ACT GCA CCC GGC ACA AAG GAG GGC                    805
Phe Pro Tyr Thr Ala Pro Gly Thr Lys Glu Gly

AAC GTG ACC TCT ACC CAC AGC GAG CTC AAC ATC                    838
Asn Val Thr Ser Thr His Ser Glu Leu Asn Ile

ACC CAG GGN NNG TGN GTA TCC CAA AAA ACC TAC                    871
Thr Gln Gly Xaa Trp Val Ser Gln Lys Thr Tyr

ACC TGC CAG GTC ACC TAT CAA GGC TTT ACC TTT                    904
Thr Cys Gln Val Thr Tyr Gln Gly Phe Thr Phe

AAA GAT GAG GCT CGC AAG TGC TCA GGT                            931
Lys Asp Glu Ala Arg Lys Cys Ser Glu

ATGGCCCCCC TGTCCCCCAG AAACCCAGAT GCGCGAGGCT                    971
CAGAGATGAG GGCCAAGGCA CGCCCTCATG CAGCCTCTCA                    1011
CACACTGCAG AG                                                  1023

TCC GAC CCC CGA GGC GTG AGC AGC TAC CTG AGC                    1056
Asp Tyr Pro Arg Gly Val Ser Ser Tyr Leu Ser

CCA CCC AGC CCC CTT GAC CTG TAT GTC CAC AAG                    1089
Pro Pro Ser Pro Leu Asp Leu Tyr Val His Lys

GCG CCC AAG ATC ACC TGC CTG GTA GTG GAC CTG                    1122
Ala Pro Lys Ile Thr Cys Leu Val Val Asp Leu

GCC ACC ATG GAA GGC ATG AAC CTG ACC TGG TAC                    1155
Ala Thr Met Glu Gly Met Asn Leu Thr Trp Tyr

CGG GAG AGC AAA GAA CCC GTG AAC CCG GTC CCT                    1188
Arg Glu Ser Lys Glu Pro Val Asn Pro Val Pro

TTG AAC AAG AAG GAT CAC TTC AAT GGG ACG ATC                    1221
Leu Asn Lys Lys Asp His Phe Asn Gly Thr Ile
```

FIG. 3B

```
ACA GTC ACG TCT ACC CTG CCA GTG AAC ACC AAT                    1254
Thr Val Thr Ser Thr Leu Pro Val Asn Thr Asn

GAC TGG ATC GAG GGC GAG ACC TAC TAT TGC AGG                    1287
Asp Trp Ile Glu Gly Glu Thr Tyr Tyr Cys Arg

GTG ACC CAC CCG CAC CTG CCC AAG GAC ATC GTG                    1320
Val Thr His Pro His Leu Pro Lys Asp Ile Val

CGC TCC ATT GCC AAG GCC CCT GGT                                1344
Arg Ser Ile Ala Lys Ala Pro Gly

GAGCCACGGG CCCAGGGGAG GTGGGCGGGC CTCCTGANCC                    1384
GGAGCCTGGG CTGACCCCAC ACCTATCCAC AGGC                           1418

AAG CGT GCC CCC CCG GAT GTG TAC TTG TTC CTG                     1451
Lys Arg Ala Pro Pro Asp Val Tyr Leu Phe Leu

CCA CCG GAG GAG GAG CAG GGG ACC AAG GAC AGA                    1484
Pro Pro Glu Glu Glu Gln Gly Thr Lys Asp Arg

GTC ACC CTC ACG TGC CTG ATC CAG AAC TTC TTC                    1517
Val Thr Leu Thr Cys Leu Ile Gln Asn Phe Phe

CCC GAG GAC ATT TCA GTG CAA TGG CTG CGA AAC                    1550
Pro Glu Asp Ile Ser Val Gln Trp Leu Arg Asn

GAC AGC CCC ATC CAG ACA GAC CAG TAC ACC ACC                    1583
Asp Ser Pro Ile Gln Thr Asp Gln Tyr Thr Tyr

ACG GGG CCC CAC AAG GTC TCG GGC TCC AGG CCT                    1616
Thr Gly Pro His Lys Val Ser Gly Ser Arg Pro

GCC TTC TTC ATC TTC AGT CGC CTG GTG GAC TGG                    1649
Ala Phe Phe Ile Phe Ser Arg Leu Val Asp Trp

GAG CAG AAA AAC AAA TTC ACC TGC CAA GTG GTG                    1682
Glu Gln Lys Asn Lys Phe Thr Cys Gln Val Val

CAT GAG GCG CTG TCC GGC TCT AGG ATC CTC CAG                    1715
His Glu Ala Leu Ser Gly Ser Arg Ile Leu Gln

AAA TGG GTG TCC AAA ACC CCC GGT AAA                            1742
Lys Trp Val Ser Lys Thr Pro Gly Lys

TGATGCCCAC CCTCCTCCCG CCGCCACCCC CCAGGGCTCC                    1782
ACCTGCTGGG GCAGGGGAGG GGGGCTGGCA AGACCCTCCA                    1822
TCTATCCTTN TCAATAAACA                                          1842
```

FIG. 3C

FIGURE 4: Canine IgE heavy chain constant region DNA sequence with translated exons 5 and 6

```
GGGGAGGGGG GGCGGGTCTG CCTTCCCCCN ACCAGCACAT                40
GAACGGCTGG ACCGGGGAGG GNTGACTGGC CGGTGCCCGC                80
A                                                          81

GAG CTA GAG CTC CAG GAG CTG TGC GCG GAT GCC                114
Glu Leu Glu Leu Gln Glu Leu Cys Ala Asp Ala

ACT GAG AGT GAG GAG CTG GAC GAG CTG TGG GCC                147
Thr Glu Ser Glu Glu Leu Asp Glu Leu Trp Ala

AGC CTG CTC ATC TTC ATC ACC CTC TTC CTG CTC                180
Ser Leu Leu Ile Phe Ile Thr Leu Phe Leu Leu

AGA GTG AGC TAC GGC GCC ACC AGC ACC CTC TTC                213
Arg Val Ser Tyr Gly Ala Thr Ser Thr Leu Phe

AAG                                                        216
Lys

GTGGGCATGC AGAGCCCCTG GCCGGGGGTG GGGGCAGCAC                256
AGAGGGAGNG AGAGGTCCCG GCAGAGCTGT CCTCACATGT                296
GCCCTCCCCC CAGGTGAAG                                       315

TGG GTA CTC GCC ACC GTC CTG CAG GTG AAG CCA                348
Trp Val Leu Ala Thr Val Leu Gln Val Lys Pro

CAG GCC GCC CAA GAC TAC GCC AAC ATC GTG CGG                381
Gln Ala Ala Gln Asp Tyr Ala Asn Ile Val Arg

CCG GCA CAG                                                390
Pro Ala Gln

TAGGCCCAGA GACACGGTGA CGAGGCCTTG CTTTCTGCCC                430
CCCNNNNNCC GGCTGAGGGC AATCTGCTGG CCCTGAGTGG                470
GAGGAGGAAA GCAGACAAAC NCAGAGGGGC CAGAGCCAGA                510
CGCCCAGCAC ACACGGATCC AGAAGCTT                             538
```

FIG. 4A

FIG. 5A Signal (secretion) sequence

```
                  M  P  A  S  M  G  G  P  A  L  L  W
 ECORI
 ------
GAATTCGGCC GCGAGATGCC TGCTTCGATG GGAGGCCCTG CCCTGCTGTG
CTTAAGCCGG CGCTCTACGG ACGAAGCTAC CCTCCGGGAC GGGACGACAC

                                        signal sequence cleavage

  +1  L  A  L  L  L  S  S  P  G  V  M  S  S  A  R  A
5451  GCTAGCGCTG CTGCTCTCCT CTCCAGGTGT CATGTCATCA GCTCGTGCCT
      CGATCGCGAC GACGAGAGGA GAGGTCCACA GTACAGTAGT CGAGCACGGA

   start exon 2
  +1  L  N  F  I  P  P  T  V  K  L  F  H  S  S  C  N  P
5501  TAAACTTCAT TCCGCCTACC GTGAAGCTCT TCCACTCCTC CTGCAACCCC
      ATTTGAAGTA AGGCGGATGG CACTTCGAGA AGGTGAGGAG GACGTTGGGG

  +1  V  G  D  T  H  T  T  I  Q  L  L  C  L  I  S  G  Y
5551  GTCGGTGATA CCCACACCAC CATCCAGCTC CTGTGCCTCA TCTCTCGCTA
      CAGCCACTAT GGGTGTGGTG GTAGGTCGAG GACACGGAGT AGAGACCGAT

  +1  V  P  G  D  M  E  V  I  W  L  V  D  G  Q  K  A
5601  CGTCCCAGGT GACATGGAGG TCATCTGGCT GGTGGATGGG CAAAAGGCTA
      GCAGGGTCCA CTGTACCTCC AGTAGACCGA CCACCTACCC GTTTTCCGAT

  +1  T  N  I  F  P  Y  T  A  P  G  T  K  E  G  N  V  T
5651  CAAACATATT CCCATACACT GCACCCGGCA CAAAGGAGGG CAACGTGACC
      GTTTGTATAA GGGTATGTGA CGTGGGCCGT GTTTCCTCCC GTTGCACTGG

  +1  S  T  H  S  E  L  N  I  T  Q  G  X  W  V  S  Q  K
5701  TCTACCCACA GCGAGCTCAA CATCACCCAG GGNNNGTGNG TATCCCAAAA
      AGATGGGTGT CGCTCGAGTT GTAGTGGGTC CCNNNCACNC ATAGGGTTTT

  +1  T  Y  T  C  Q  V  T  Y  Q  G  F  T  F  K  D  E
5751  AACCTACACC TGCCAGGTCA CCTATCAAGG CTTTACCTTT AAAGATGAGG
      TTGGATGTGG ACGGTCCAGT GGATAGTTCC GAAATGGAAA TTTCTACTCC

  +1  A  R  K  C  S  E  S  D  P  R  G  V  S  S  Y  L  S
5801  CTCGCAAGTG CTCAGAGTCC GACCCCCGAG GCGTGAGCAG CTACCTGAGC
      GAGCGTTCAC GAGTCTCAGG CTGGGGGCTC CGCACTCGTC GATGGACTCG

  +1  P  P  S  P  L  D  L  Y  V  H  K  A  P  K  I  T  C
5851  CCACCCAGCC CCCTTGACCT GTATGTCCAC AAGGCGCCCA AGATCACCTG
      GGTGGGTCGG GGGAACTGGA CATACAGGTG TTCCGCGGGT TCTAGTGGAC
```

FIG. 5A

```
  +1   L  V  V   D  L  A  T   M  E  G   M  N  L   T  W  Y
5901   CCTGGTAGTG GACCTGGCCA CCATGGAAGG CATGAACCTG ACCTGGTACC
       GGACCATCAC CTGGACCGGT GGTACCTTCC GTACTTGGAC TGGACCATGG

  +1 R  E  S  K   E  P  V   N  P  V  P   L  N  K   K  D  H
5951   GGGAGACCAA AGAACCCGTG AACCCGGTCC CTTTGAACAA GAAGGATCAC
       CCCTCTNGTT TCTTGGGCAC TTGGGCCNGG GAAACTTGTT CTTCCTAGTG

  +1  F  N  G  T   I  T  V   T  S  T   L  P  V  N   T  N  D
6001   TTCAATGGGA CGATCACAGT CACGTCTACN CTGCCAGTGA ACACCAATGA
       AAGTTACCCT GCTAGTGTCA GTGCAGATGN GACGGTCACT TGTGGTTACT

  +1   W  I  E   G  E  T  Y   Y  C  R   V  T  H   P  H  L
6051   CTGGATCGAG GGCGAGACCT ACTATTGCAG GGTGACCCAC CCGCACCTGC
       GACCTAGCTC CCGCTCTGGA TGATAACGTC CCACTGGGTG GGCGTGGACG

  +1 P  K  D  I   V  R  S   I  A  K  A   P  G  K   R  A  P
6101   CCAAGGACAT CGTGCGCTCC ATTGCCAAGG CCCCTGGCAA GCGTGCCCCC
       GGTTCCTGTA GCACGCGAGG TAACGGTTCC GGGGACCGTT CGCACGGGGG

  +1   P  D  V  Y   L  F  L   P  P  E   E  E  Q  G   T  K  D
6151   CCGGATGTGT ACTTGTTCCT GCCACCGGAG GAGGAGCAGG GGACCAAGGA
       GGCCTACACA TGAACAAGGA CGGTGGCCTC CTCCTCGTCC CCTGGTTCCT

  +1   R  V  T   L  T  C  L   I  Q  N   F  F  P   A  D  I
6201   CAGAGTCACC CTCACGTGCC TGATCCAGAA CTTCTTCCCC GCGGACATTT
       GTCTCAGTGG GAGTGCACGG ACTAGGTCTT GAAGAAGGGG CGCCTGTAAA

  +1 S  V  Q  W   L  R  N   D  S  P  I   Q  T  D   Q  Y  T
6251   CAGTGCAATG GCTGCGAAAC GACAGCCCCA TCCAGACAGA CCAGTACACC
       GTCACGTTAC CGACGCTTTG CTGTCGGGGT AGGTCTGTCT GGTCATGTGG

  +1   T  T  G  P   H  K  V   S  G  S   R  P  A  F   F  I  F
6301   ACCACGGGGC CCCACAAGGT CTCGGGCTCC AGGCCTGCCT TCTTCATCTT
       TGGTGCCCCG GGGTGTTCCA GAGCCCGAGG TCCGGACGGA AGAAGTAGAA

  +1   S  R  L   E  V  S  R   V  D  W   E  Q  K   N  K  F
6351   CAGCCGCCTG GAGGTTAGCC GGGTGGACTG GGAGCAGAAA AACAAATTCA
       GTCGGCGGAC CTCCAATCGG CCCACCTGAC CCTCGTCTTT TTGTTTAAGT

  +1 T  C  Q  V   V  H  E   A  L  S  G   S  R  I   L  Q  K
6401   CCTGCCAAGT GGTGCATGAG GCGCTGTCCG GCTCTAGGAT CCTCCAGAAA
       GGACGGTTCA CCACGTACTC CGCGACAGGC CGAGATCCTA GGAGGTCTTT
```

end exon 4

FIG. 5B

```
   +1 W  V  S  K  T  P  G  K  *
6451  TGGGTGTCCA AAACCCCCGG TAAATGATGC CCACCCTCCT CCCGCCGCCA
      ACCCACAGGT TTTGGGGGCC ATTTACTACG GGTGGGAGGA GGGCGGCGGT

6501  CCCCCCAGGG CTCCACCTGC TGGGAGGGAG GGGGGCTGGC AAGACCCTCC
      GGGGGGTCCC GAGGTGGACG ACCCTCCCTC CCCCCGACCG TTCTGGGAGG

                          polyadenylation signal
6551  ATCTGTCCTT GTCAATAAAC ACTCCAGTGT CTGCTTGGAG CCCTGGGCAC
      TAGACAGGAA CAGTTATTTG TGAGGTCACA GACGAACCTC GGGACCCGTG

6601  ACCCATTTCT TGGGGGTGGG CAGGGTTGCA GAGCAGGGAT GTCTTGGCAC
      TGGGTAAAGA ACCCCCACCC GTCCCAACGT CTCGTCCCTA CAGAACCGTG
```

FIG. 5C